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# MICE: THEIR BREEDING AND REARING FOR SCIENTIFIC PURPOSES<sup>1</sup>

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## I. INTRODUCTION

NOTWITHSTANDING many shortcomings mice have contributed much to the advancement of science and the service of mankind. To realize this we have but to recall that it was by crossing the white with the gray mouse that Mendel's Law of Inheritance was first found to apply to the animal kingdom (1); that from a study on mice some of the earliest concepts of immunity were obtained (2); and that from experiments now in progress on them an insight is being gained into the nature of cancer (3). These and similar experiments indicate something of the scope to which these animals have been put.

The ease with which white mice can be handled makes them, in many ways, preferable for experimentation to other and larger rodents. But, owing to a widespread notion that they are difficult to rear under laboratory conditions, their usefulness has been greatly curtailed.

The method usually employed in the breeding of mice has been what we may term extensive. By this I mean that many animals are kept from which to obtain offspring. I have set myself the task of breeding mice intensively, that is, of keeping relatively few, but of keeping these under conditions which will insure their productivity. It is the purpose of this paper to describe the way in which this was done.

## II. THE INTENSIVE BREEDING OF MICE

### A. *Detrimental Factors*

1. *Marked Fluctuations in Temperature.*—Probably no single factor is more likely to be overlooked than that mice, to produce to the best advantage, require an equable temperature. While they can withstand extremes

<sup>1</sup>From the Zoological Laboratory, University of California.

of heat or cold, such extremes are not conducive to their productivity. At 35° C. I have found breeding to be greatly impeded, and at a temperature as low as 2° C. the young born are subject to a number of mortal ills which practically prevent their reaching maturity. But a constant temperature of either of the above extremes is not so detrimental as is great fluctuation in temperature. A mouse taken from favorable conditions and subjected to daily fluctuations of from 30° C. to 2° C. soon becomes a different animal physiologically. The fur which was sleek and glossy roughens, the exposed veins in the ears and tail darken, and the animal is readily reduced to a condition which, if prolonged, not infrequently terminates in death. If after having reached this condition, however, the animal be promptly restored to equable conditions of temperature, its fur becomes sleek, signs of anæmia disappear and the mouse regains its normal health and vigor often with surprising rapidity.

2. *Parasitism*.—If mice, even under the most favorable conditions of temperature, become badly parasitized breeding ceases and unless they are ridded of the parasites the adult mice as well the young fall victims to this pest. To test the effects of parasitism, I have taken mice from fresh stock, kept under excellent conditions, and have placed them in infested nests, with the result that in a few days the mice became sluggish, and many sooner or later died.

When the two factors—parasitism and wide fluctuations in temperature—are combined, the animals, especially the young, die in great numbers.

### *B. Factors Essential to Intensive Breeding*

1. *Construction and Equipment of Cases*.—In general, where a number of mice are to be kept together, the wire and wood cases described by Yerkes (4) has been much used. But for intensive breeding I have found it better to keep few mice in a case, and to keep these under better conditions of sanitation than is possible in the above case.

I know of no better plan to insure sanitation than to construct cases which will offer little surface upon which

dirt may collect, and which at the same time will make evident that which has accumulated. Such a case should be made with a perforated bottom and should be pro-

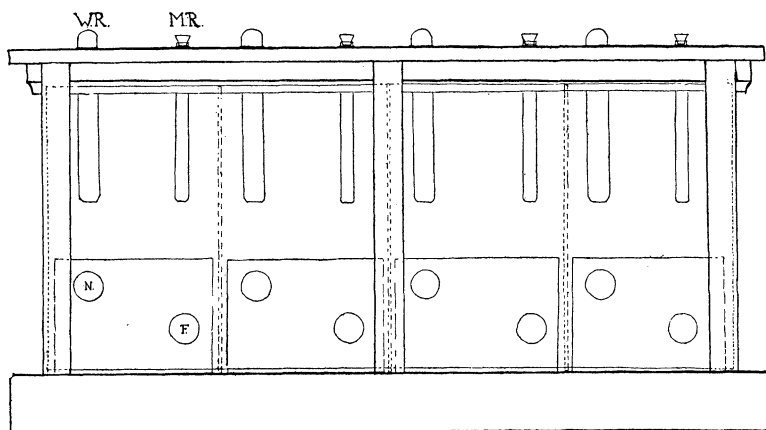


FIG. 1. FRONT VIEW OF CASE. *W.R.*, water receptacle; *M.R.*, milk receptacle; *N*, entrance to nest; *F*, opening to food.

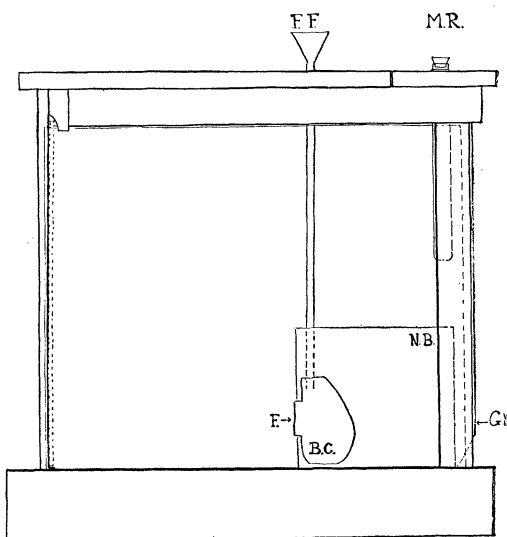


FIG. 2. END VIEW OF CASE. *FF*, food funnel; *M.R.*, milk receptacle; *N.B.*, nest box; *Gb*, galvanized iron back (near its bend); *F*, entrance to food cup *B.C.*

vided with sides of glass. Briefly described,<sup>2</sup> the case that I have constructed consists of a framework of light wood, a back of galvanized iron and sides, front and par-

<sup>2</sup> For detail see description accompanying drawings of the case.

tition of  $10 \times 12$  glass. The top is made of screen wire; the bottom of  $\frac{1}{4}$  inch wire mesh (hardware cloth).

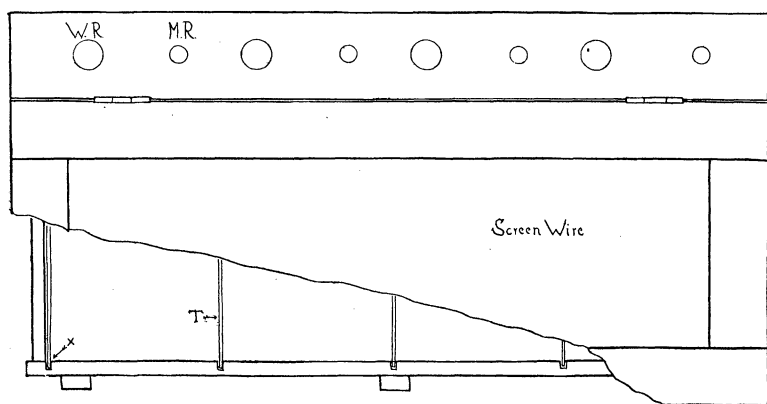


FIG. 3. TOP OF CASE, WITH A PART REMOVED TO SHOW HOW THE GLASS PARTITIONS AND ENDS (X) ARE FIXED INTO THE FRAME. WR., water receptacles; T, a bent piece of tin covering the upper end of the glass to increase the height of the partition and at the same time to cover the sharp edge of the glass.

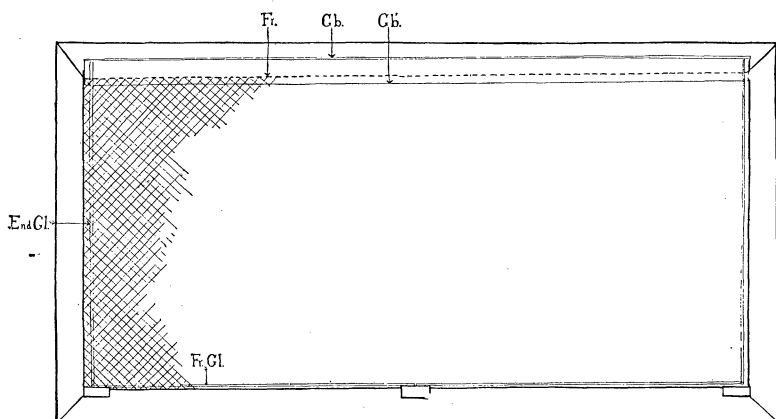


FIG. 4. BOTTOM VIEW OF THE CASE. The wire mesh is partly drawn in to show its relation to the end (*End. gl.*) and front glass (*Fr. gl.*), and to the galvanized iron back (*Gb'*). *Fr.*, frame.

From a sanitary point of view too much emphasis can hardly be placed upon the construction of the bottom. By using  $\frac{1}{4}$  inch wire mesh through which waste material will readily fall, I have succeeded in providing a case which in a large measure is self cleaning. The cases thus constructed are placed on a long trough-like table, cov-

ered with galvanized iron, which drains into a sink. By flushing off the top at intervals, the waste is easily disposed of.

While the use of glass in case building may imply an increase in the cost of construction, yet in the above plan it is believed that this cost has been reduced to a minimum, and that a case has been provided which in addition to its quality for observation assures unusual conditions of sanitation.

A point of great importance in the construction of such a case is that the galvanized iron back and the side and front glasses rest on the mesh-bottom so that waste material in falling through does *not* strike the framework of the case. It will be seen from a view of the bottom (Fig. 4) that the back of the case is bent inward an inch from its base so as to come well out on the mesh bottom, and that the sides and front are placed well over the line of the framework of the bottom.

2. *Nest Boxes and Nesting Materials*.—I have followed with satisfactory results the largely used plan of having a winter and a summer nest box. The winter nest box is made by cutting a chalk box to three-fourths size. This is filled two-thirds full of nesting material. The summer nest is a small sized box similarly furnished.

Various materials have been tested for nesting, to many of which objections can be made. Cotton although warm, retains odor and at the same time offers a more serious objection in that the young often become entangled in it, and are thus permanently injured or even killed. Excelsior I have found to make a good nest if lined with some sort of soft material, as, for example, crude floss. One of the most satisfactory materials which I have tried for nesting is the shredded paper used in the packing of china. If this can not be procured at the china store it may be prepared by cutting up any kind of soft paper.

3. *Food Receptacles and Food*.—I have tried various kinds of food receptacles, most of which have given little or no satisfaction. The difficulty of keeping the food reasonably clean in open food cans is so great that some

sort of device for its protection is essential. The plan that I have found most successful has been to place bird cups in the nest box so that the mouse can procure the food through an opening in the front of the box. The cups are further provided with an apperture in the top through which, without opening the case, food may be added. This method of feeding, by preventing the mouse from running over the food, holds in abeyance the spread of disease.

The most satisfactory food that I have used for mice is wheat, added to which is a small amount of stale bread. These, together with milk—which is given by a modification of the method below described for water—constitute the daily and constant diet. Occasionally sunflower seed and a few leaves of lettuce may well be given for a change.

4. *Water Receptacles*.—The inverted bottle which is now in general use for supplying water has done much to eradicate ills resulting from a bad water supply. By this device a large quantity of water can be provided which is a great advantage in general cultures. In many kinds of experiments, however, it is desirable to keep water in greater purity than is possible even by this method. To do this I have found a device which is strikingly simple and at the same time singularly effective in that it keeps the water in contact only with glass. My plan consists in closing the end of a specimen-tube (or test-tube) in a frame so that the opening is just large enough to retain the water drop when the tube is filled and inverted in the case.<sup>3</sup> These inverted tubes are inserted through holes made in the top of the case and are prevented from falling through by means of small rubber bands placed around the closed ends of the tubes. The tube may then be removed and refilled without opening the case, the refilling being done from a siphon bottle.

The addition of these devices for food and water to a mesh-bottom case I have found to aid much in the inten-

<sup>3</sup> The tube for milk, instead of having one end closed permanently as in the test-tube, has the end closed with a rubber stopper, the advantage of this being the ease with which such an open tube upon removal of the stopper can be cleaned.

sive breeding of mice. But to rear mice successfully further requires a practical knowledge of their breeding habits.

### III. BREEDING HABITS OF MICE

Copulation in mice is a well-defined act which usually follows only upon the persistent efforts of the male. It is marked by a period of union which lasts for several seconds (ten to twenty-five) and is followed by an interval of more or less complete rest.

Practical questions for the breeder are: 1. When will copulation occur—that is, when is the period of heat? 2. What is the duration of this period? 3. How often does the period of heat recur? To the last of these questions my experience can offer no answer; and to the second my observations add little. In one case, however, after a double copulation had been observed in the evening copulation again took place on the following morning. The fact that the beginning of heat is shown in some as early as five hours after parturition and is delayed in others as long as thirty-six hours thereafter makes it difficult to determine with exactness the duration of this period.

As to the first question—When may copulation be secured?—two periods can be determined with considerable accuracy. One of these closely succeeds parturition, the other follows upon a period of rest.

In the first case, if the female has given birth to young, copulation will usually take place, if she is put with the male, within from five to twenty-four hours.<sup>4</sup> In my own experience I have found that the greater number of births take place in the early morning, and that copulation will, in such a case, occur from seven to eight o'clock in the evening. The female is not invariably in heat at this time, however, as has been shown by a considerable number of cases which I have observed.

The second period in which copulation may be expected is after a mother has gone through an interval of rest,

<sup>4</sup> This is seen to correspond roughly to the time of ovulation in mice, as shown by Long (5), the period given being from  $14\frac{1}{2}$  to  $28\frac{1}{2}$  hours after parturition.



either after having suckled her young or after having lost them. She will then ordinarily copulate within a few days after having been put with the male. The following representative table of ten consecutive cases emphasizes this point.

TABLE I

Example	Put with Male	Young Born	Interval Elapsing, Days.
1	April 2	April 25	23
2	April 5	April 26	21
3	April 12	May 4	22
4	April 19	May 11	22
5	April 29	May 21	22
6	May 14	June 6	23
7	May 18	June 11	24
8	May 26	June 24	29
9	May 27	June 18	22
10	June 11	July 3	22

Out of the above ten cases in which the females had not suckled young for some time, and then were put with males, nine cases of copulation evidently resulted early, since the young were born within an interval only a little greater than the normal period of gestation following a rest (that is, 20 days) (6).

The eighth case, although unusual, has been further accentuated by more recent data which show that the female may remain with the male for long periods without becoming pregnant. This is not conclusive evidence, however, that an unsuccessful copulation may not have taken place within that time. In fact, I have found that in a surprising number of cases copulation does not result in fertility. As an example of this may be cited 25 consecutive cases which I observed, 10, or 36 per cent., of which resulted in infertility.

In interpreting this sterility I was at first inclined to believe that it was due entirely to the females, but since then I have found males in many cases unproductive. Some of these were useless for breeding because they were practically unresponsive, rarely if ever copulating; others, although among the most active males, proved unfertile. The following example may be given to show such a case.

TABLE IIa  
RECORD OF ♂ No. 6

No. of ♀	6	9	2	5	6	4	2	1	14	10
Date of copulation	12/3	12/7	12/8	12/14	12/23	12/27	12/30	1/6	4/3	4/19
Result. . . . .	Fert.	Infer.	Fert.	Fert.	Infer.	Fert.	Infer.	Infer.	Infer.	Infer.

In the above table is given the record of one of the most active males that I have yet had. The record, however, shows that, although mated with vigorous females, only 40 per cent. of the copulations resulted in offspring. The table further shows that after a rest from January to April infertility is still shown. At this later period the male had become inactive, so that it was difficult to secure a copulation.

A relatively high index of fertility is shown in the record of a male designated as No. 5.

TABLE IIb  
RECORD OF ♂ No. 5

No. of ♀	3	4	3	5 <sup>b</sup>	3	4	9	6	1	2
Date of copulation.	12/3	12/7	12/28	1/3	1/17	1/21	3/8	3/13	3/13	4/6
Result. . . . .	Fert.	Fert.	Fert.	Infer.	Infer.	Fert.	Fert.	Fert.	Fert.	Fert.

Number 5 when mated with females equally as active as those with which No. 6 was mated gave 80 per cent. of fertility. Within this series were also two other copulations by the same mouse, but since these were with female No. 4, which always proved fertile, they were eliminated and only those counted which were entirely comparable with those of No. 6.

It may be said that both males and females are found which have a low index of fertility. Intensive breeding requires that these be eliminated and that those be selected the copulations of which result in a high percentage of fertility.

When fertility does result from a copulation, the ensuing period is of singular interest to the investigator. This I have discussed in a former paper<sup>6</sup> in which I have

<sup>5</sup> Seriously ill after the birth of her young.

<sup>6</sup> Loc. cit.

shown that the period of gestation depends upon the state of the female. If the pregnant female is not suckling young, parturition with but rare exceptions takes place on the twentieth day after copulation. If she is suckling on the other hand, the period varies with the number of young suckled. Thus, for example, if the mother be suckling five during gestation she may be expected to go about twenty-five days; if ten thirty days.

Parturition, which terminates the period of gestation is normally of brief duration, even in case a large litter is born. But this is not invariable, for I have observed cases in which labor was prolonged, and some in which unaided birth was impossible.

We are inclined to believe that in mankind the difficulty of giving birth, which not infrequently results in the death of the mother, is due to the artificialities of civilized life. But here we find the same stern fact emphasized in a type remotely removed from any such influence.

#### IV. REARING OF THE YOUNG

The most hazardous time in the life of a mouse is the first few days of its existence. Born helpless and naked it is dependent upon the mother not alone for nourishment but for warmth as well. Some mothers at this time are most solicitous for their young, building elaborate nests for them and giving such care to the young as to tide them over this early period. Others there are that not only withhold the requisite care, but which at this time prove the most serious menace. Some of these bundle their young away in the nesting to die; while others in bad conditions openly destroy them. This singular and, so far as I am aware, unexplained phenomenon of destroying the offspring, is carried to a high pitch in the case of mice. Under unfavorable conditions of temperature, nesting and the like, I have seen three or four litters destroyed in succession. Miller (7) in a study of the brown rat shows that this destructiveness in the rat is carried to even a greater extent than in the case of the mouse.

If the young escape the perils of the first few days they usually grow rapidly and, at the end of a few months, reach maturity. There is a disease, however, which at the end of the second week may attack the young, leaving them emaciated or, when more severe, killing them in great numbers.

Between birth and maturity four well-defined stages occur. To know these is often of practical service to the breeder for the determination of age, sex and the like.

*The first stage* is that in which the newly-born young have a peculiarly red and transparent skin through which is seen the stomach white with milk. Following this at the end of the sixth or seventh day a *second stage* is evident in which the body is covered with flaky scales of dandruff—the forerunners of a coat of silky fur. A *third important stage*, which I have designated as the early stage for distinguishing sex<sup>7</sup> is usually shown on the ninth or tenth day, at which time the mammæ in the young females appear. These can be observed for an interval up to the thirteenth or fourteenth day, at which time the fur usually obscures them.

Determination of sex after the body is covered with fur, for example at the time of weaning, is often difficult. Because of this I have found it advantageous at the end of the third period to mark the young females by clipping a tuft of fur at the root of the tail, so that later, when they are to be mated, no difficulty is found in distinguishing with certainty males from females.

*The fourth period*, on the fourteenth day, is characterized by the advent of sight. This like all other periods shows slight variation. While in a few cases I have found the eyes to open as early as the thirteenth day, in others, equally normal in other respects, I have found them to be delayed until the fifteenth and even the sixteenth day. The regularity with which this period occurs, however, is a sufficiently exact criterion to make it an index of age.

From the fourteenth day to the twenty-first, the date

<sup>7</sup> This does not mean that sex can not be determined earlier than this period. As a matter of fact, sex can be determined at birth, this, however, is difficult and less certain than to determine it at the third period.

at which the young should be weaned, no definite change, except increase in size, is shown.

With the period of sexual maturity we may count the cycle of development complete. This does not imply, however, that growth ceases at this time. The time at which mice reach sexual maturity varies greatly. While I have had some mice to pair at six weeks, this is rather unusual. It has been my experience that under ordinary circumstances both the males and females reach sexual maturity in the second or third month. From this time on for the next few months the mice are in the prime of the reproductive period, beyond which, at the end of ten to twelve months of age, activity diminishes and, for purposes of breeding, the mouse is of little further service.

#### V. PRACTICAL SUMMARY

The ease with which white mice can be handled makes them in many cases preferable for experimentation to other and larger rodents, but their usefulness has been greatly curtailed because of a wide-spread notion that they are difficult to rear under the conditions of the laboratory.

The purpose of this paper is to give an intensive method by which I have been able to rear them in abundance. By "intensive" I mean that relatively few mice are kept from which to breed, and that these are kept under conditions which insure productivity.

Especially detrimental to intensive breeding are parasitism and marked fluctuations in temperature. Hot air heat, the temperature ranging from 20° to 25° C., has proved most satisfactory. Heat from an oil stove when continued for a considerable length of time proved unsatisfactory.

Mice which are badly parasitized are useless for breeding purposes. Various kinds of sprays and powders are used to rid them of the parasites. Some of these, however, I have used with disastrous results. Parasitism may be best prevented by using a cage that is readily cleaned. My cages are washed weekly with hot water to

which is added "gold-dust" and a small amount of petroleum; the nest-boxes, into which the mice are put at the time of cleaning, are partly closed and then removed from the case.

Essential to intensive breeding is an adequately equipped case. A most essential requisite is a wire mesh bottom through which waste material readily falls. If such a case be placed on a table with a trough-top lined with galvanized iron, waste matter can easily be drained into a sink. For nests the case is provided with three-fourth sized chalk boxes filled two-thirds full of shredded paper. Food (wheat) is procured by the mice through an opening in the front of the nest box from bird cups placed inside of the box. These are filled from the outside without opening the case. Water may be kept in excellent condition in test-tubes which are closed sufficiently to retain the water drop when the tube is inverted in the case; these are filled from a siphon bottle.

A knowledge of breeding habits is of great importance in intensive breeding. Copulation in mice is a well-defined act, which lasts from ten to twenty-five seconds and is followed by a more or less complete rest. It normally takes place on the day that a litter is born. In females that have gone through a period of rest it will usually occur a few days after the female has been put with the male. Copulation may or may not result in fertility. By a selection of males and females with a high index of fertility the number of offspring may be greatly increased.

The period which a non-suckling mother carries her young is a few hours short of twenty days. A mother suckling young, on the other hand, carries her litter twenty plus the number that she is suckling. Thus a mother suckling five will go practically  $(20+5)$  twenty-five days, while one suckling ten may be expected to run  $(20+10)$  thirty days.

In rearing the young it is well to remember that the greatest mortality results within the first two or three days of life. At this time the young must be kept warm. Under bad conditions the mother may destroy them.

From birth to maturity mice pass through several well-defined stages which to the breeder are of importance for the determination of age, sex and the like. These are 1 an early stage in which the skin is peculiarly red so that through it may be seen the stomach white with milk; 2 a second stage at six to seven days in which the body is covered with flakes of dandruff; 3 a stage at nine or ten days in which the mammae appear in the females. This I have designated as the stage for the early determination of sex; 4 on the fourteenth day a stage at which the eyes open.

At twenty-one days the young should be weaned. From this time on slight change is shown except increase in size until sexual maturity is reached. This usually occurs in the second or third month. From this time up to the end of ten months or a year of age the mouse is in the height of the breeding period; beyond this time, for purposes of breeding, the mouse is usually of little further service.

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